A Primer Extension Assay to use for cattle Genotype Assisted Selection and Parentage and Traceability Analysis

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Motivation

• Most of the beef populations are of small census and application of selection programs are costly and difficult (e.g. application of genomic selection).
• Only a few tens of mutations at different genes have been associated so far to different traits (milk, meat, growth performance or coat colour in cattle).
• There is a need of parentage testing to guarantee genealogic information for selection and conservation purposes.
• The existence of meat labels in many small census beef cattle breeds, makes traceability a need to allow its guarantee.

Aims

The development of a low-medium throughput genotyping assay, to generalise its use in bovine for:
• Marker Assisted Selection.
• Pedigree analysis.
• Traceability analysis.
Using both validated trait associated mutations and mutations at candidate genes.

Strategy

• **Samples:** 436 animals corresponding to the following breeds: Pasiega, Lidia, Asturiana de los Valles, Casina, Avileña Negra-Ibérica, and Pirenaica Spanish breeds; Danish Red, Holstein, and Simmmental Danish breeds; Limousin, and Charolais French breeds; and Aberdeen Angus, Jersey, South Devon, and Highland English breeds.
• **Genes:** 70 SNPs tested in 24 *trait associated* genes *:* related to milk production (CSN3, DGAT1, BLACT, FADS1, PPARGC1A, GH), tenderness (CAPN3, CAST, LOX), muscular growth (CHRNE, GHR, CRH, GDF8, UCP2, POMC), marbling (DGAT1, RORC, LEP, TG), and coat colour (MC1R, c-KIT, SILV, TYR, TYRP1), and 39 SNPs in non validated c*andidate genes.*
• **Genotyping method:** Capillary Primer-Extension Assay.

Results

• Seventy polymorphisms belonging to 61 genes have been genotyped by Capillary Primer-Extension Assay and allele frequencies were calculated.

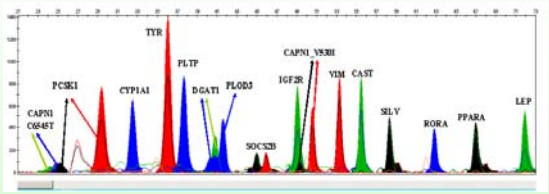

Figure 1. ABI3130 sequencer electropherogram corresponding to one of the four multiplexes presented, analyzed using GeneMapper v4.0 (Applied Biosystems)

•Estimation of the power for parentage analysis and animal identification on four different sets of polymorphisms (Table 1):
i) The whole loci tested except for the *Amelogenin* gene (69)[1].
ii) The polymorphisms associated to different traits (31 SNPs in 24 genes)[2].
iii) The SNPs with minor allele frequency (MAF) exceeding a 0.3 threshold at least in one breed (46)[3].
iv) A panel which combines the polymorphisms from the two latter groups (53)[4].

	69 loci[1]	31 loci[2]	46 loci[3]	53 loci[4]
H_e	0.30	0.31	0.39	0.35
PIC	0.24	0.25	0.31	0.28
Excl P1[5]	0.986	0.880	0.981	0.982
Excl P2[6]	0.999	0.999	0.999	0.999
PI[7]	$7.13 \cdot 10^{-19}$	$3.22 \cdot 10^{-9}$	$2.17 \cdot 10^{-16}$	$7.32 \cdot 10^{-17}$
PSI[8]	$5.08 \cdot 10^{-10}$	$4.21 \cdot 10^{-5}$	$8.57 \cdot 10^{-9}$	$5.00 \cdot 10^{-9}$

[5] Combined exclusion probability when only one parent was genotyped
[6] Combined exclusion probability when the parent pair genotypes were available
[7] Combined probability identity
[8] Combined probability sib identity

Table 1. Parentage analysis and animal identification powers

Conclusions

• The assay developped here should allow performing Genotype Assisted Selection, paternity testing and traceability analysis at an affordable cost and low effort.
• To reach the statistical power desirable to combine the three objectives, we propose the 53 SNPs sub-set (which includes all SNPs from 24 trait associated genes plus 22 SNPs corresponding to candidate genes) resolved in two Multiplex-Primer Extension Assays.
• The flexibility of the Capillary Primer-Extension Assay allows the introduction of future polymorphisms which may be of interest in small beef cattle populations, thereby implementing the genetic signature at any time.